Brief Communications

Transient Upregulation of Postsynaptic IP_3-Gated Ca Release Underlies Short-Term Potentiation of Metabotropic Glutamate Receptor 1 Signaling in Cerebellar Purkinje Cells

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Synaptic plasticity lasting ~100 s has been suggested to function as a temporary buffer for neural information. One example of this was reported by Batchelor and Garthwaite (1997), who found that a slow metabotropic glutamate receptor 1 (mGluR1)-evoked EPSP produced by burst stimulation of cerebellar parallel fiber–Purkinje cell synapses could be potentiated by a conditioning stimulus consisting of prior activation of climbing fiber synapses (or injection of depolarizing current) with a delay of up to 90 s. What is the molecular basis of the signal that spans this temporal gap? Here, we show that mGluR1-evoked slow EPSCs evoked by parallel fiber burst test stimuli show a similar form of short-term potentiation (mGluR1-STP) and that this phenomenon is also observed when parallel fiber bursts are replaced by pressure pulses of an exogenous mGluR1 agonist. Ca imaging experiments revealed that cytosolic Ca levels returned to baseline within several seconds after conditioning depolarization, indicating that this cannot underlie mGluR1-STP. To test the hypothesis that transient upregulation of inositol-1,4,5-trisphosphate (IP_3)-gated Ca release underlies this phenomenon, we used local photolytic uncaging of IP_3 to deplete IP_3-gated Ca stores. IP_3 uncaging in the interval between conditioning depolarization and the test pulse produced a complete blockade of mGluR1-STP, as did blockade of IP_3 receptors with heparin. When Ca transients evoked by IP_3 uncaging were used as a test stimulus, conditioning depolarization produced a large STP of Ca response amplitudes. These data suggest that transient upregulation of postsynaptic IP_3-gated Ca signaling constitutes a novel form of short-term synaptic plasticity.

Key words: **Purkinje cell; mGluR1; potentiation; IP_3; Ca stores; cerebellum**

Introduction

It has been suggested that long-term use-dependent changes in synaptic strength underlie at least a portion of the engram for persistent memory storage. However, in addition to more permanent forms of memory, it appears as if neural computation also requires a "scratchpad" or temporary buffer for information storage on the time scale of 1–100 s. Several examples of synaptic plasticity on this time scale have been found. One interesting example involves a heterosynaptic type of signal integration. Batchelor and Garthwaite (1997) made microelectrode current-clamp recordings from Purkinje cells in slices of adult rat cerebellum and evoked EPSPs by delivering test volleys to the parallel fibers. They found that whereas single volleys only evoked a fast AMPA receptor mediated EPSP, brief bursts evoked both fast EPSPs and, after that, a slow EPSP that was sensitive to an antagonist of metabotropic glutamate receptor 1 (mGluR1). Purkinje cells receive excitatory glutamatergic synapses from both the parallel and the climbing fibers. The latter is a single axon that forms ~1500 release sites on a Purkinje cell and consequently produces a massive excitation, which triggers the opening of dendritic voltage-sensitive Ca channels, causing a widespread Ca influx. When a single climbing fiber volley was delivered before the parallel fiber burst test pulse, the amplitude of the mGluR1-evoked slow EPSP was potentiated, whereas the amplitude of the AMPA receptor-evoked fast EPSP was unchanged. This phenomenon, which we shall call mGluR1-STP, could be recorded with climbing fiber-parallel fiber burst intervals of up to ~90 s. It is likely that dendritic Ca influx produced by climbing fiber activation triggered mGluR1-STP, because it could be induced when climbing fiber stimulation was replaced with either injection of somatic depolarizing current sufficient to evoke Ca spiking or by dendritic photolysis of caged Ca (Batchelor and Garthwaite, 1997).

mGluR1 is localized to the perisynaptic region of parallel fiber–Purkinje cell synapses (Lopez-Bendito et al., 2001). As a consequence, the glutamate released by single parallel fiber volleys, which is subject to diffusional dilution and reuptake into glial cells and neurons, is insufficient to activate perisynaptic mGluR1. However, burst stimulation produces a larger and longer glutamate transient that can overcome diffusion and reuptake and thereby activate perisynaptic mGluR1 (Tempia et al., 1998; Brasnjo and Otis, 2001). mGluR1 is coupled to activation of phospho-

Received Nov. 15, 2006; revised March 13, 2008; accepted March 17, 2008.

This work was supported by National Institutes of Health (NIH) Grants MH051106 and MH068830 and the Develbiss Fund (D.J.L.), NIH Grant DA10309 (P.F.W.), and Korea Research Foundation Grant KRF-2005-015-E00033 (S.J.K.). We thank Roland Bock for skillful technical assistance and members of the Worley, Linden, and Kim laboratories for helpful suggestions.

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DOI:10.1523/JNEUROSCI.0284-08.2008

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lipase Cβ through G_q (Hartmann et al., 2004). Phospholipase C produces diacylglycerol and inositol-1,4,5-trisphosphate (IP_3), the latter of which binds IP_3 receptors on the endoplasmic reticulum to evoke Ca mobilization from internal stores (Finch and Augustine, 1998; Takechi et al., 1998). A second limb of mGluR1 signaling involves activation of transient receptor potential canonical 1 (TRPC1)-containing ion channels in the plasma membrane, producing the cation influx that mediates the mGluR1-EPSC (Kim et al., 2003).

What is the molecular basis of the signal that spans the 90 s gap between climbing fiber-evoked Ca influx and the parallel fiber burst test stimulus? In other words, what is the molecular basis of the "short-term memory" in the mGluR1-STP? We have performed whole-cell voltage-clamp recording of Purkinje cells together with confocal Ca uncaging and spot photolysis of IP_3 to address this question.

Materials and Methods

Parasagittal slices of the cerebellar vermis (250 μm thick) were prepared from P17–P20 Sprague Dawley rats using a vibrating tissue slicer and ice-cold standard artificial CSF (ACSF) containing 124 mM NaCl, 2.5 mM KCl, 1 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, and 20 mM D-glucose, bubbled with 95% O_2 and 5% CO_2. After a recovery period of 30 min at 35°C, the slices were placed in a submerged chamber that was perfused at a rate of 2 ml/min with ACSF supplemented with either 100 μM picrotoxin or 5 μM GABAzine to block $GABA_A$ receptors. The visualized whole-cell patch-clamp technique and Ca imaging experiments were performed at 30–32°C. The recording electrodes (resistance 2–3 MΩ) were filled with a solution containing 135 mM Cs-methanesulfonate, 10 mM CsCl, 10 mM HEPES, 4 mM Na_2ATP, 0.4 mM Na_3GTP, and 0.2 mM EGTA (substituted with 0.2 mM Oregon Green BAPTA-1 in Ca imaging experiments), pH 7.25. Currents were filtered at 1 kHz, digitized at 5 kHz, and acquired using pClamp 9.0 software. For parallel fiber stimulation, standard patch pipettes were used, which were filled with ACSF and placed in the middle third of the molecular layer. Synaptic responses were evoked every 30 s using 12–16 μA pulses (100 μs duration). When burst stimulation was used, the interpulse interval was 10 ms. In some experiments, membrane currents were evoked by agonist application using a pressure application system (10 psi, 10–50 ms pulse duration).

A confocal laser-scanning head attached to an upright microscope (Axioskop 2FSmot; 40× water-immersion objective; numerical aperture, 0.8; Zeiss, Thornwood, NY) was used to acquire fluorescence images during the whole-cell recordings. For photolytic uncaging experiments, the internal saline was supplemented with NPE-IP_3 (300 μM). Uncaging was produced by directing the 355 nm output of a frequency-tripled Nd:YVO_4 laser operating at 100 kHz (DPSS, Santa Clara, CA; power at the laser head, 1.1 W; pulse duration, 50 ns) into the epifluorescence train of the confocal microscope using a single-mode fiber (200–600 μm diameter) and coupling optics. Light was directed to the objective by reflection from a 400 nm dichroic mirror. Uncaging test pulses (5–15 ms duration) were originated by Clampex 9.0 software and controlled by a combination of laser switching and activation of a mechanical shutter. Confocal Ca imaging was started at least 20 min after establishment of whole-cell configuration to allow for dendritic perfusion. The image data were analyzed with ImageJ. There was considerable variation both between cells and between dendritic locations in a single cell regarding UV-evoked Ca transients. In some loci, UV-evoked Ca transients required prior priming depolarization (duration of 50–100 ms to 0 mV). In others, moderated amplitude responses were evoked without priming. Only loci that showed the latter behavior were used to construct Figure 4.

All group data are shown as mean ± SEM. Comparisons were made using Student's *t* test. All drugs were purchased from Sigma (St. Louis, MO) except for CPCCOEt, GABAzine, NBQX, SKF 96365, and cyclopiazonic acid (CPA; Tocris Bioscience, Ellisville, MO), and Oregon Green BAPTA-1 and NPE-IP_3 (Invitrogen, Carlsbad, CA).

Results

Whole-cell voltage-clamp recordings were made from cerebellar Purkinje cells in sagittal brain slices. The ACSF was supplemented with a $GABA_A$ receptor antagonist and NBQX (5 μM), a dose designed to produce an ~90% blockade of AMPA receptors. A stimulating electrode placed to activate parallel fibers was used to deliver brief bursts as test stimuli (10 pulses, 100 Hz). With V_{hold} set to −70 mV, this evoked a cluster of 10 fast EPSCs followed by a slow EPSC (Kim et al., 2003). Previous work has shown that this slow EPSC requires an mGluR1–G_q–TRPC1 signaling cascade (Kim et al., 2003; Hartmann et al., 2004). After a parallel fiber burst test pulse, a conditioning stimulus consisting of a somatic command depolarization to 0 mV for 100 ms was delivered, and then an additional parallel fiber burst test pulse was given at various intervals from 10 to 180 s (Fig. 1*A*). The conditioning stimulation produced an STP of the slow mGluR1-evoked EPSC (mGluR1-STP), but had no effect on the fast EPSC mediated by AMPA receptors. This differential effect argues against either nonspecific changes in electrical properties of the Purkinje cell or changes in the release of glutamate from parallel fibers as the basis of mGluR1-STP. Analysis of mGluR1-STP across a range of intervals revealed that it was largest at the shortest interval recorded (160.9 ± 8.8% at 10 s interval; $n = 7$) and that it was no longer operative at an interval of 180 s (99.7 ± 2.7%; $n = 7$). The time course of mGluR1-STP was fit with a single exponential with a time constant of 54 s (Fig. 1*B*).

These experiments were repeated by replacing parallel fiber burst stimulation in NBQX with a brief micropressure pulse of ACSF supplemented with glutamate (50 μM) and DHPG (100 μM). This produces separable fast and slow inward currents, the latter of which are blocked by mGluR1 antagonists (Kim et al., 2003). When conditioning depolarization was followed by a glutamate/DHPG pulse at an interval of 10 s, a selective STP of the slow current was produced (Fig. 1*C*, top) (132.0 ± 4.5%; $n = 7$). When the conditioning stimulation was changed to a single climbing fiber shock in current-clamp mode, a selective STP of the slow potential was produced (Fig. 1*C*, middle) (147.2 ± 5.7%; $n = 18$). These experiments confirm the findings of Batchelor and Garthwaite (1997) and extend them in several important ways. They show that mGluR1-STP is an increase in the mGluR1-evoked current itself rather than a secondary effect on voltage-sensitive ion channels. Furthermore, they show that mGluR1-STP can be revealed even when synaptic glutamate release is bypassed through application of exogenous agonist, arguing strongly for a solely postsynaptic locus of expression.

One candidate mechanism for the persistent signal underlying mGluR1-STP is the tail of the depolarization-evoked cytosolic Ca transient. To address this possibility, we included the Ca indicator Oregon Green BAPTA-1 (200 μM) and the caged compound NPE-IP_3 (300 μM) in the internal saline and performed whole-cell recording together with Ca imaging using a laser-scanning confocal microscope. Parallel fiber burst stimulation produced a slow, localized Ca transient in the region of dendrite underlying the stimulating electrode (Fig. 2*A*). This Ca transient did not begin until after the last fast EPSC. The peak amplitude of the Ca transient was 59.2 ± 12.7% dF/F ($n = 11$). This Ca transient was completely blocked by the mGluR1 antagonist CPCCOEt (100 μM; data not shown). The 100-ms-long conditioning stimulus evoked a large and widespread Ca transient (319.5 ± 37.3% dF/F; $n = 11$) with a single-exponential decay (time constant, 0.33 ± 0.03 s; $n = 11$). Ten seconds after the onset of the depolarizing conditioning stimulus, the evoked Ca transient was completely

decayed (−0.2 ± 0.9% d*F*/*F*; $n = 11$). The second parallel fiber burst test stimulus delivered at this point showed mGluR1-STP as reflected in the amplitude of the slow EPSC (178.0 ± 11.0% of predepolarization; $n = 11$) and also a similar increase in the amplitude of the associated Ca transient (control, 253.9 ± 31.9% of predepolarization; $n = 11$). This increase in the Ca transient may reflect increased Ca influx via TRPC1-containing channels, increased Ca mobilization from internal stores, or both. These measurements argue against the notion that cytosolic free Ca is a signal underlying mGluR1-STP.

One possibility is that transient upregulation of postsynaptic IP_3-gated Ca signaling adjacent to synaptically activated mGluR1 could support mGluR1-STP. If this is correct, then depletion of IP_3-gated Ca stores should block this phenomenon. To this end, the previous stimulation and recording protocol (parallel fiber burst-pause-depolarization-pause-parallel fiber burst) (Fig. 2*A*) was repeated a few minutes later in the same cell with one addition. Six seconds after the conditioning depolarization, a brief pulse from an ultraviolet laser was used to produce photolysis of caged IP_3 at the same spot that showed Ca transients evoked by parallel fiber burst test stimuli (Fig. 2*B*). This produced a large, brief cytosolic Ca transient (104.1 ± 4.5% d*F*/*F*; $n = 11$). When the second parallel fiber burst test stimulus was delivered 10 s after depolarization onset, mGluR1-STP was blocked as indexed by the amplitude of both the slow EPSC (control, 178.0 ± 11.0% of predepolarization; after UV pulse, 114.0 ± 6.8% of predepolarization; $p < 0.001$; $n = 11$) and the amplitude of the slow Ca transient (control, 253.9 ± 31.9% of predepolarization; after UV pulse, 118.1 ± 12.3% of predepolarization; $p < 0.001$; $n = 11$) (Fig. 2*C*). As a control experiment, UV uncaging of IP_3 was interposed between parallel fiber burst test pulses without conditioning depolarization. In this control condition, UV exposure induced Ca transients (37.1 ± 11.6% d*F*/*F*; $n = 7$), but had no effect on the amplitude of subsequent parallel fiber burst-evoked slow EPSCs (100.5 ± 4.2% of control) and Ca transients (91.6 ± 7.9% of control) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). CPA (25 μM), an endoplasmic reticulum Ca-ATPase pump inhibitor, completely blocked the Ca transient evoked by uncaging IP_3 (6.5 ± 1.5% of control; $p < 0.05$; $n = 6$) (supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

If transient upregulation of postsynaptic IP_3-gated Ca signaling underlies mGluR1-STP, then blockade of IP_3 receptors would be expected to attenuate mGluR1-STP. To test this hypothesis, we included the IP_3 receptor antagonist heparin (6 mg/ml) in the internal saline. In this configuration, conditioning depolarization failed to induce mGluR1-STP as reflected in the amplitude of the slow EPSC (control, 171.1 ± 10.9%; $n = 8$; $p < 0.001$; heparin, 122.3 ± 2.9%; $n = 8$; $p < 0.001$) or the associated slow Ca transient (control, 219.4 ± 23.4%; $n = 8$; $p < 0.01$; heparin, 104.4 ± 2.4%; $n = 8$; $p < 0.05$) (Fig. 3*A*). In a separate set of experiments, mGluR1-STP was assayed using the slow mGluR1-mediated current evoked by glutamate/DHPG test pulses (control pipette, 132.0 ± 4.5%; $n = 7$; heparin pipette, 93.0 ± 4.5%; $n = 7$; $p < 0.001$) or the associated slow Ca transient (control pipette, 300.2 ± 67.2%; $n = 7$; heparin pipette, 126.7 ± 18.5%; $n = 7$; $p < 0.05$) and was also found to be blocked by postsynaptic application of heparin. What is the origin of the remaining Ca transient accompanying the mGluR1 slow EPSC in heparin-loaded Purkinje cells? We found that in this condition, SKF

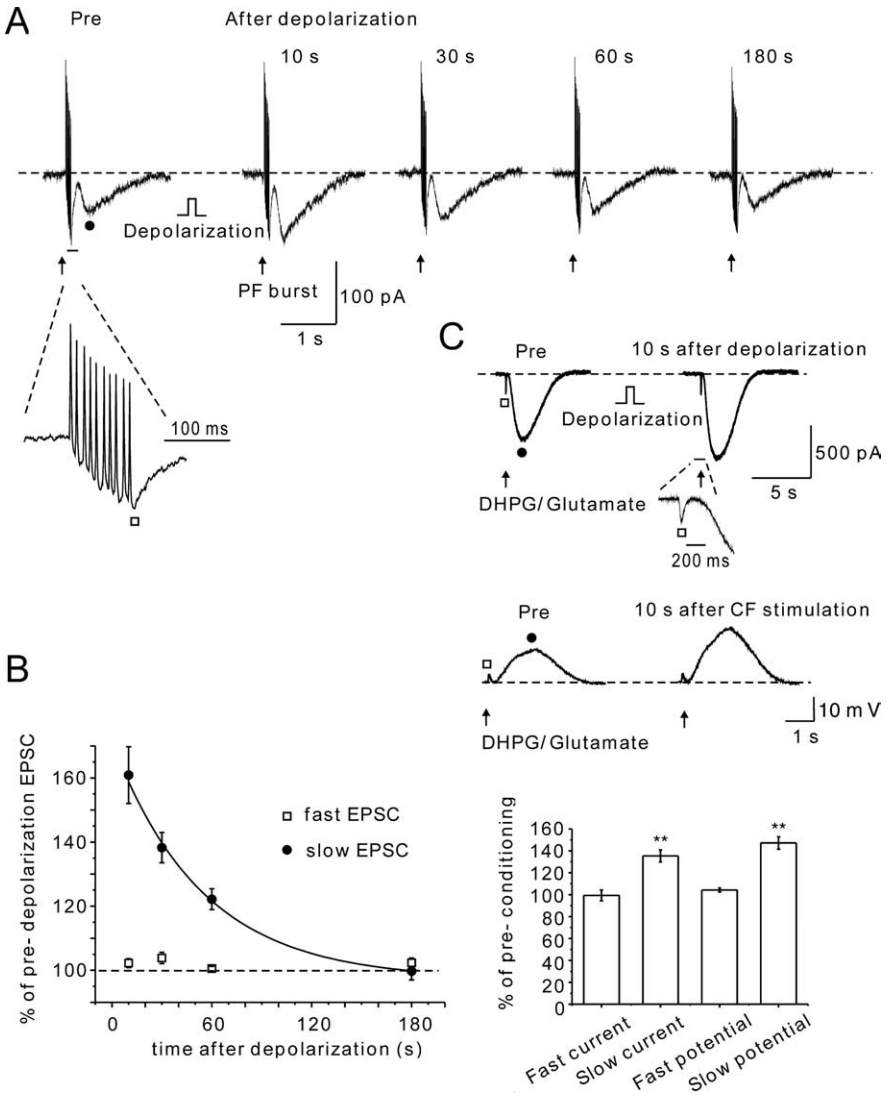

Figure 1. mGluR1-STP is expressed postsynaptically. ***A***, Fast (open square) and slow (closed circle) EPSCs were produced by a parallel fiber (PF) burst (10 pulses, 100 Hz). Representative current traces from four different trials are shown before (Pre) and 10, 30, 60, and 180 s after a conditioning stimulus (a somatic command depolarization to 0 mV for 100 ms). ***B***, The time course of the amplitude of the mGluR1-evoked slow EPSC was fit with single-exponential decay. ***C***, Fast (open square) and slow (filled circle) responses were evoked by micropressure pulses (10 psi, 20 ms duration) of ACSF containing DHPG (100 μM) and glutamate (50 μM). Top traces, Voltage-clamp experiments for which representative current traces are shown before and 10 s after conditioning depolarization. Bottom traces, Current-clamp experiments performed in a different population of cells. Representative voltage traces are shown before (Pre) and 10 s after a conditioning stimulus consisting of a single climbing fiber (CF) shock. Bar graph, Population data are expressed as the percentage of preconditioning peak amplitude of currents, and potentials are presented as mean ± SEM. $^{**}p < 0.01$.

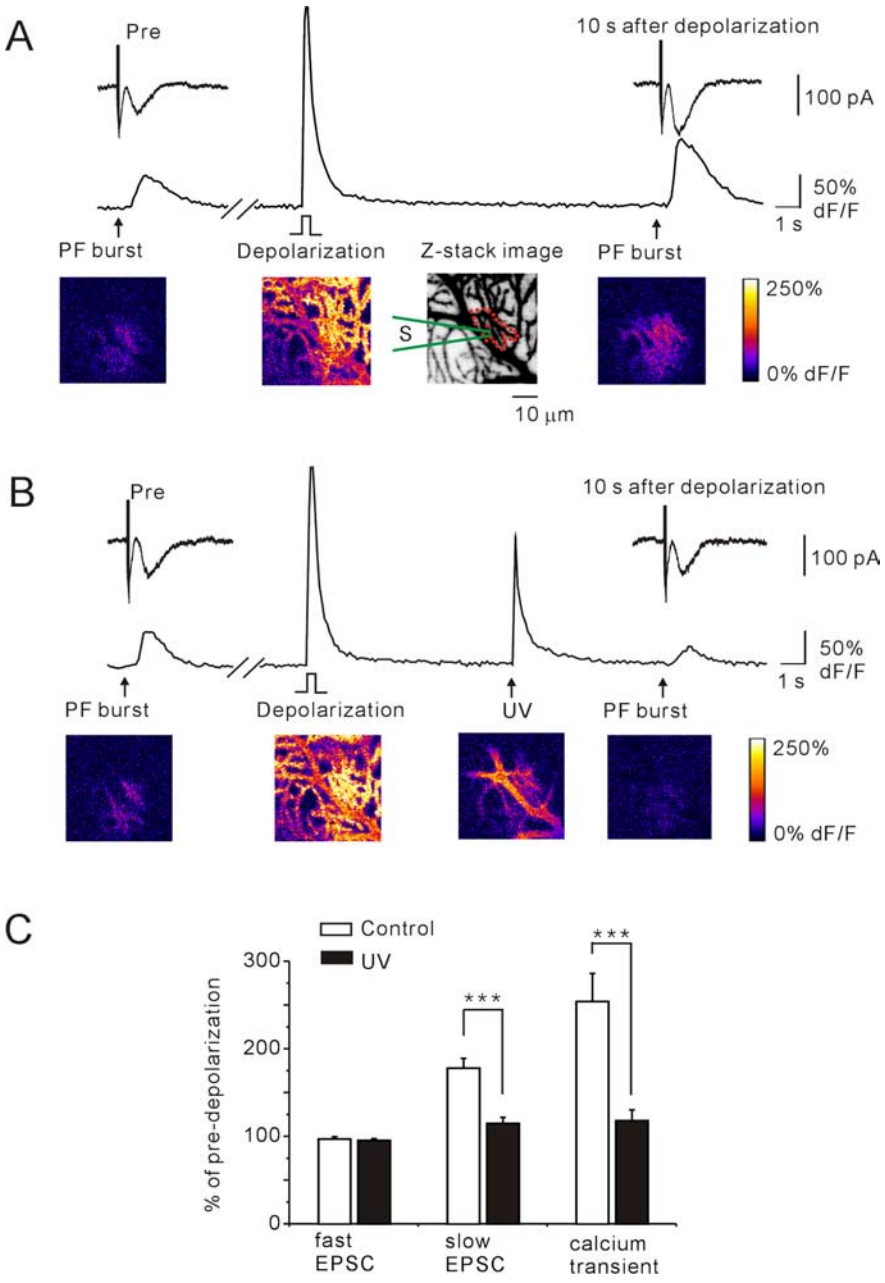

Figure 2. mGluR1-STP is blocked by IP_3 uncaging during the conditioning–test interval. ***A***, Purkinje cells were loaded with both Ca indicator (Oregon Green BAPTA-1) and caged-IP_3 (NPE-IP_3). Parallel fiber (PF) bursts produced fast and slow EPSCs and accompanying localized Ca transients. Representative current and Ca traces are shown before (Pre) and 10 s after a 100-ms-long conditioning depolarization. Pseudocolor images of the Purkinje cell dendrites that show the peak Ca transient derived from a series of 15 images acquired at 10 Hz illustrate the relative increase in Ca-dependent fluorescence (d*F*/*F*) evoked by a parallel fiber burst and by the conditioning depolarization. A projected *z*-stack image illustrates detailed dendritic morphology surrounding the stimulating electrode location (S; shown in green). Ca traces were derived from a region of interest (ROI) drawn to encompass a segment of dendrite underlying the stimulating electrode (outlined in red). ***B***, The same stimulation and recording protocol as in ***A*** was repeated a few minutes later in the same cell. A 15 ms pulse from an ultraviolet laser was delivered to produce photolysis of caged IP_3. The UV spot was ~20 μm in diameter and approximately centered on the ROI as well. ***C***, Population data for the experiments shown in ***A*** and ***B*** are expressed as the percentage of predepolarization peak amplitude of EPSCs and Ca transients. Data are mean ± SEM. ***$p < 0.001$.

96365, a TRPC antagonist, completely blocked the slow EPSC and associated Ca transient (slow EPSC, 8.8 ± 2.0% of baseline; $n = 6$; $p < 0.001$; Ca transient, 4.2 ± 0.5%; $n = 6$; $p < 0.001$) (supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

If postsynaptic IP_3-gated Ca signaling is transiently upregulated by conditioning depolarization, then it should be possible to query the status of this signaling cascade at various time points after depolarization. To accomplish this, we perfused cells with caged IP_3 and Ca indicator and performed spot uncaging of IP_3 and Ca imaging (Fig. 4; supplemental Fig. 4, available at www.jneurosci.org as supplemental material). An IP_3 uncaging test pulse was delivered to determine a baseline response, and this was followed 10 s later by conditioning depolarization, and then a second IP_3 uncaging pulse was delivered at intervals of 10–180 s. At an interval of 10 s, a very large facilitation of the evoked Ca transient was observed (553.9 ± 169.7% of predepolarization; $n = 6$), whereas at 180 s, no facilitation was evident (101.8 ± 2.1% of predepolarization; $n = 6$). The decay of the IP_3-evoked Ca transient facilitation was fit with a single exponential, yielding a time constant of 24 s. This indicates that dendritic IP_3-gated Ca signaling is indeed transiently upregulated after conditioning depolarization.

Discussion

The overall goal of these experiments was to illuminate the expression mechanism of mGluR1-STP. There are several main findings. First, mGluR1-STP can be recorded under voltage-clamp conditions as a specific potentiation of the mGluR1-evoked slow EPSC but not the fast AMPA receptor-mediated EPSC. Furthermore, the same phenomenon can be observed when glutamate/DHPG test pulses are used in place of parallel fiber bursts. These results confirm and extend the original report of Batchelor and Garthwaite (1997), which used current-clamp recording and microelectrodes, and argue strongly for postsynaptic locus of expression for mGluR1-STP. Furthermore, these results argue for a direct effect on the mGluR1 current as opposed to voltage-sensitive ion channels triggered by slow depolarization. Second, the cytosolic free Ca transient evoked by the conditioning stimulus decays with a time constant of 0.33 s, and although it is likely to trigger mGluR1-STP, it cannot be the signal that underlies the persistence of mGluR1-STP for >60 s. Third, mGluR1-STP can be blocked by two different manipulations: depletion of IP_3-gated Ca stores during the conditioning depolarization–test pulse interval by local IP_3 uncaging and postsynaptic IP_3 receptor blockade with internal application of heparin. Fourth, the conditioning depolarization used to trigger mGluR1-STP also produces a large transient potentiation of the Ca transient evoked by local uncaging of IP_3. This phenomenon, like mGluR1-STP, decays to baseline values between 60 and 180 s.

We suggest that conditioning depolarization produces a tran-

sient upregulation of IP_3-gated Ca signaling that lasts for ~90 s and that it is this phenomenon that underlies the temporal span of mGluR1-STP. There are several caveats that should be sounded in regards to the present findings. First, it is not straightforward to dissociate the loading state of dendritic Ca stores from the efficacy of the IP_3 receptors that access those stores. For example, the results in Figures 3 and 4 could result from a transient increase in the efficacy of IP_3 receptors without a change in the filling status of the stores. However, the experiment in Figure 2*B*, in which IP_3 uncaging during the interval between the conditioning depolarization and the test pulse blocked mGluR1-STP, is harder to reconcile with a transient increase in IP_3 receptor efficacy as the underlying mechanism. To do so, one would have to hypothesize that IP_3 uncaging produced a powerful downregulation of IP_3 receptor function lasting for at least 4 s, the interval between the caged IP_3 pulse and the test stimulus. Although we believe that transient store filling is the most parsimonious explanation for the results herein, it should also be emphasized that an explanation based on store filling and another based on IP_3 receptor efficacy are not mutually exclusive.

A second caveat involves the exact relationship between mGluR1-STP as reflected in the amplitude of the slow mGluR1-evoked EPSC (and the slow mGluR1-evoked Ca transient) and the transient potentiation of Ca transients evoked by IP_3 uncaging. Ten seconds after the conditioning depolarization, the slow EPSC was approximately twofold larger, and the associated slow Ca transient was ~2.5-fold larger. When this measurement was made using IP_3 uncaging as the test stimulus, the IP_3-evoked Ca transient was ~5.5-fold larger. Although all of these measures decayed to baseline in the period between 60 and 180 s, the time constant fit to mGluR1-STP of the slow EPSC was 54 s, whereas that for the decay of the IP_3-evoked Ca transient was 24 s. This suggests that the relationship between these two measures may be nonlinear or involve intervening steps. In particular, the mGluR1-EPSC may be modulated both by Ca mobilization from stores and by Ca influx.

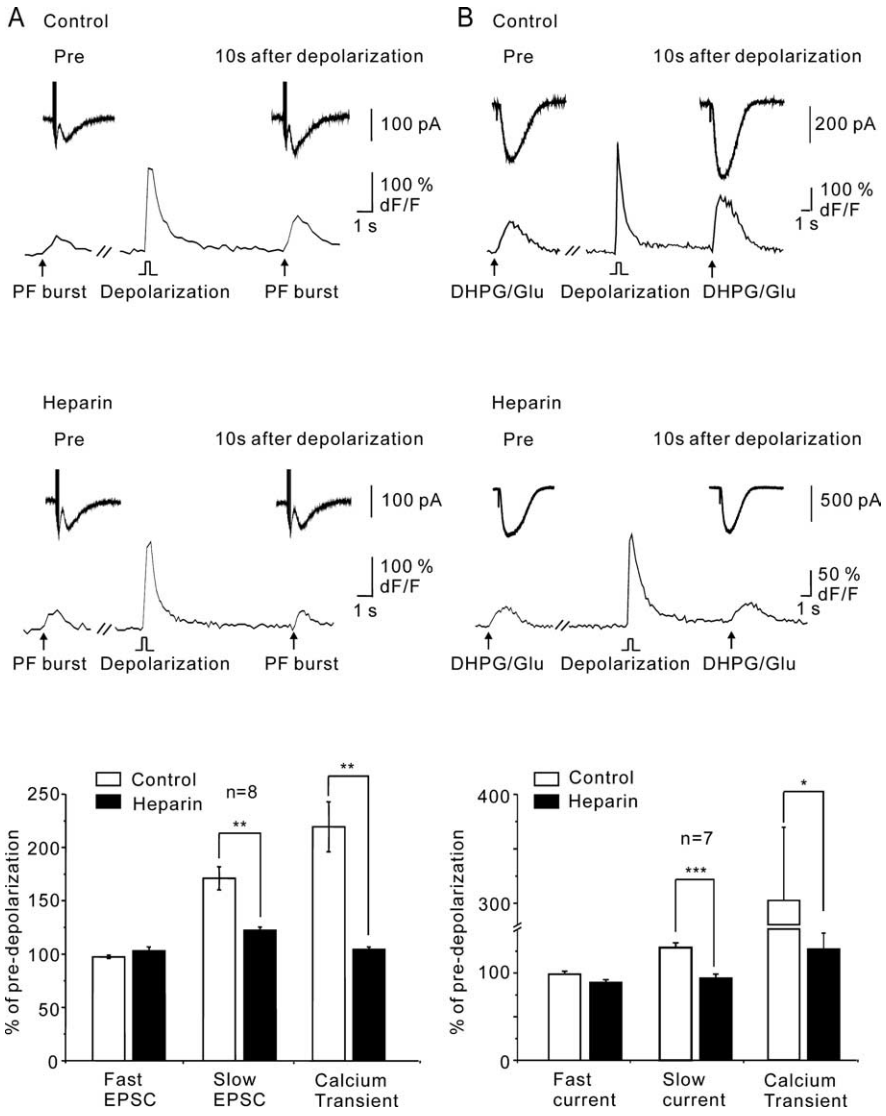

Figure 3. Postsynaptic application of an IP_3 receptor antagonist blocks mGluR1-STP. ***A***, Purkinje cells were loaded with either normal internal saline or internal saline supplemented with the IP_3 receptor antagonist heparin (6 mg/ml), perfused for ~40 min. Parallel fiber (PF) bursts produced fast and slow EPSCs and accompanying localized Ca transients. Representative traces are shown before (Pre) and 10 s after the conditioning depolarization, which failed to induce mGluR1-STP in heparin-loaded cells. Population data for control and heparin are compared as the percentage of predepolarization peak amplitude of EPSCs and Ca transient and are presented as mean ± SEM. $^{**}p < 0.01$. ***B***, Fast and slow inward currents and the associated cytosolic Ca transient were evoked by micropressure pulses of external solution containing DHPG (100 μM) and glutamate (50 μM). Representative traces are shown before (Pre) and 10 s after the conditioning depolarization, which failed to induce mGluR1-STP in heparin-loaded cells. Population data are compared as the percentage of predepolarization peak amplitude of evoked currents and Ca transients and are presented as mean ± SEM. $^{*}p < 0.05$; $^{***}p < 0.001$.

How does a transient increase in the status of dendritic IP_3-gated Ca signaling result in mGluR1-STP as reflected in both the slow EPSC and its associated Ca transient? The blockade of mGluR1-STP by the IP_3 receptor antagonist heparin indicates that IP_3 receptors are required for this process. However, beyond that we can only speculate. There are several components of mGluR1 signaling that are known to be highly Ca sensitive, including mGluR1 itself, phospholipase Cβ, and the type 1 IP_3 receptor (Bezprozvanny et al., 1991; Miyakawa et al., 2001; Okubo et al., 2004). It will be useful for future studies to address these potential mechanisms.

Activation of mGluR1 in Purkinje cells is known to trigger both short- and long-term forms of synaptic plasticity. Long-term depression of both parallel fiber (Aiba et al., 1994; Conquet et al., 1994) and climbing fiber (Hansel and Linden, 2000) synapses requires mGluR1 induction. Similarly, transient depression of glutamate release at parallel fiber–Purkinje cells synapses evoked by brief bursts requires a signaling cascade involving mGluR1 activation, synthesis of endocannabinoids, and ligation of CB1 receptors on parallel fiber terminals (Brown et al., 2003; Maejima et al., 2005; Kawamura et al., 2006). This suggests the possibility that the induction threshold for these phenomena

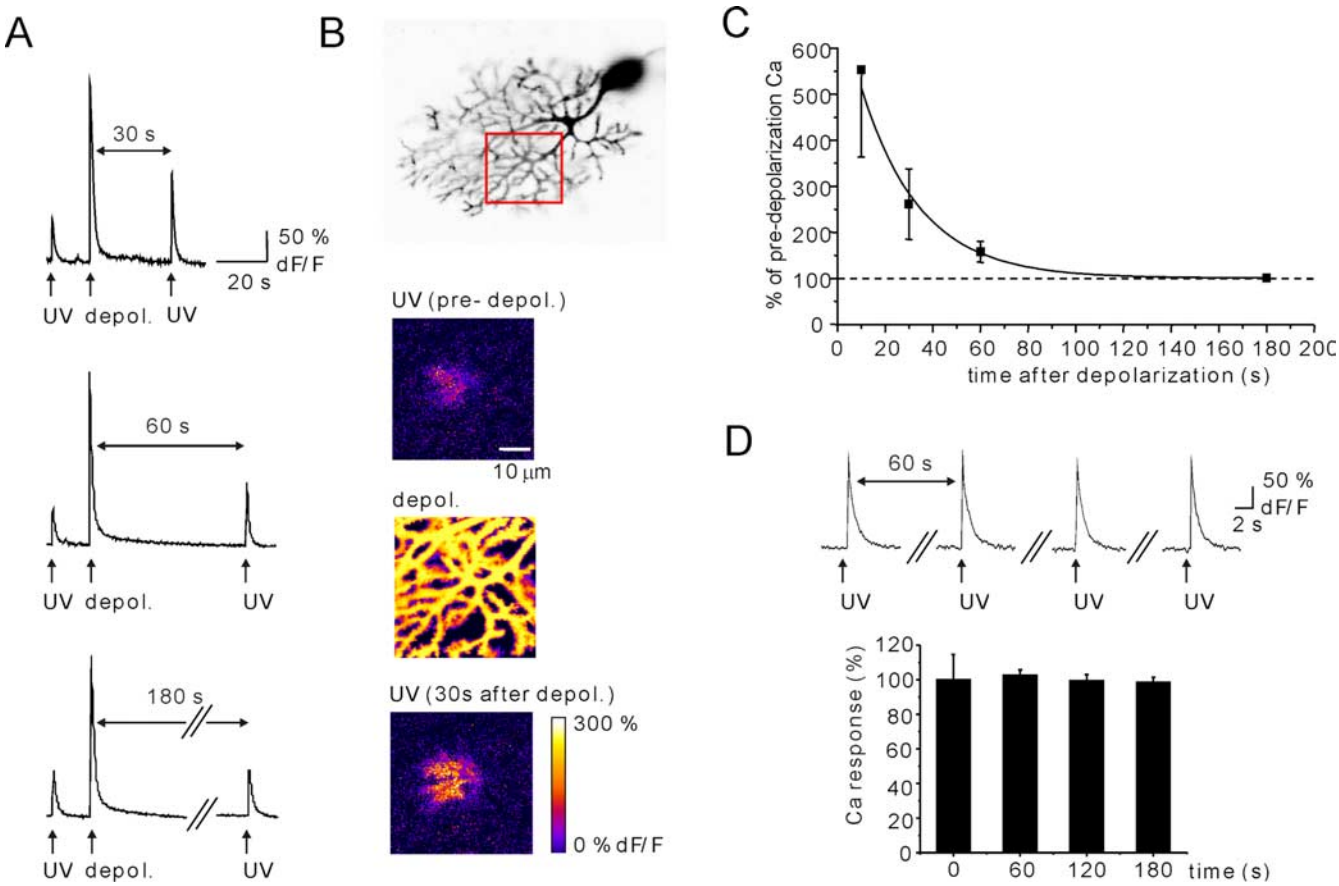

Figure 4. Conditioning depolarization produces a transient potentiation of Ca mobilization from stores evoked by local dendritic IP_3 uncaging. ***A***, A 5 ms pulse from an ultraviolet laser (spot diameter, ~20 μm) produced a cytosolic Ca transient in a cell loaded with caged IP_3. A second pulse was delivered 10, 30, 60, and 180 s after the conditioning stimulus (a somatic command depolarization to 0 mV for 100 ms). This conditioning depolarization induced short-term potentiation of the IP_3-evoked Ca transient. A rest period of >180 s was imposed between each interval testing trace to avoid response rundown. ***B***, The stimulated region of the dendrite (shown by the red rectangle in the top panel) was used to measure Ca signals during UV-induced IP_3 uncaging and conditioning depolarization. As in Figure 2 *A*, Ca traces were derived from a region of interest encompassing a segment of dendritic shaft directly underlying the UV spot. ***C***, The decay of the UV-induced Ca transient, shown here as population data ($n = 6$), was fit with a single exponential. ***D***, A control experiment to show that repeated UV pulses delivered at 60 s intervals evoke Ca responses that do not run down over four repetitions ($n = 8$).

might be lowered for ~90 s after conditioning depolarization (or climbing fiber activation).

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